

1 **Histone deacetylase 3 facilitates TNF α -mediated NF- κ B activation through suppressing**

2 **CTSB induced RIP1 degradation and is required for host defense against bacterial infection**

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31 **This file includes**

32 Fig. S1-S4

33 Table S1-S2

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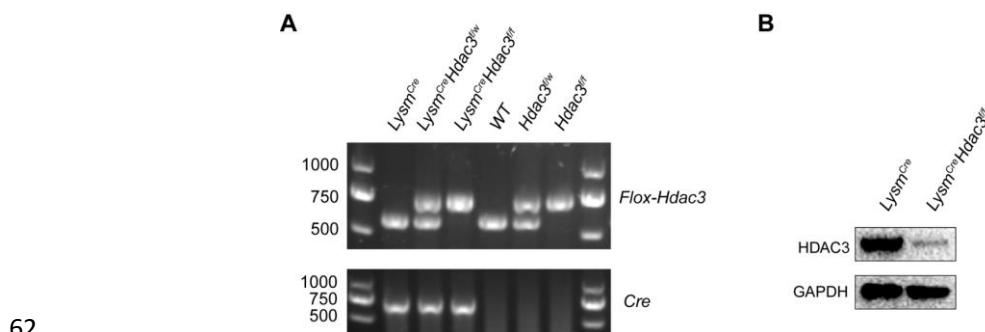
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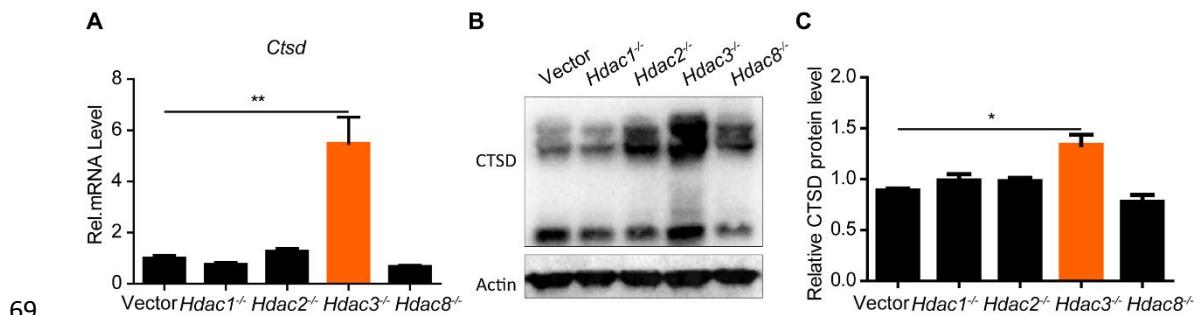
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61 **Figure S1**62 **Fig. S1. Identification of mice of different genotypes.**

63 **A** PCR analysis of the expression of *Flox-Hdac3* and *Cre* gene in *Lysm^{Cre}Hdac3^{fl/fl}*,
64 *Lysm^{Cre}Hdac3^{fl/w}*, *Lysm^{Cre}*, *Hdac3^{fl/fl}*, *Hdac3^{fl/w}* and WT mice. **B** Western blot analysis of HDAC3 of
65 BMDMs from *Lysm^{Cre}* and *Lysm^{Cre}Hdac3^{fl/fl}* mice.

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67 **Figure S2**68 **Fig. S2. HDAC3 deficient macrophages have elevated expression of Ctsd.**

69 **A** PCR analysis of *Ctsd* in Vector, *Hdac1^{-/-}*, *Hdac2^{-/-}*, *Hdac3^{-/-}*, *Hdac8^{-/-}* RAW264.7 cells. **B-C**
70 Western blot analysis of CTSD in Vector, *Hdac1^{-/-}*, *Hdac2^{-/-}*, *Hdac3^{-/-}*, *Hdac8^{-/-}* RAW264.7 cells.
71 Data are representative of three independent experiments and showed as mean \pm SEM. *P<0.05
72 and **P<0.01.

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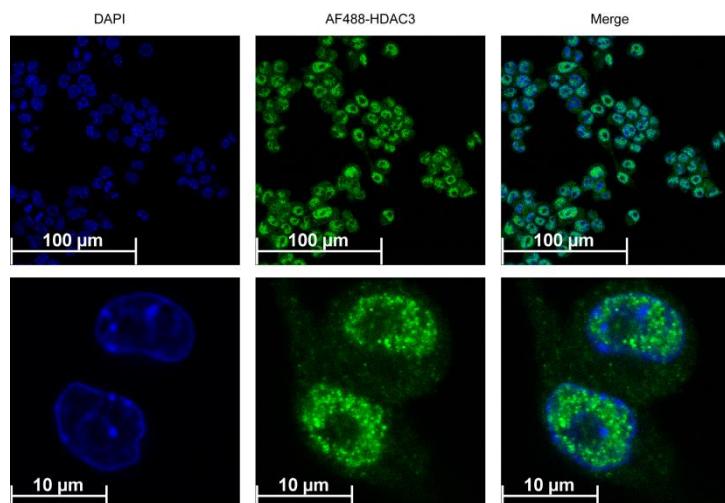
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82 **Figure S3**



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84 **Fig. S3. HDAC3 is mainly located in the nucleus.**

85 Confocal micrographs of RAW264.7 stained with DAPI (blue, DNA) and antibodies against
86 HDAC3 (AF488). Scale bar: 100/10 μm . Figures are representative of three independent
87 experiments.

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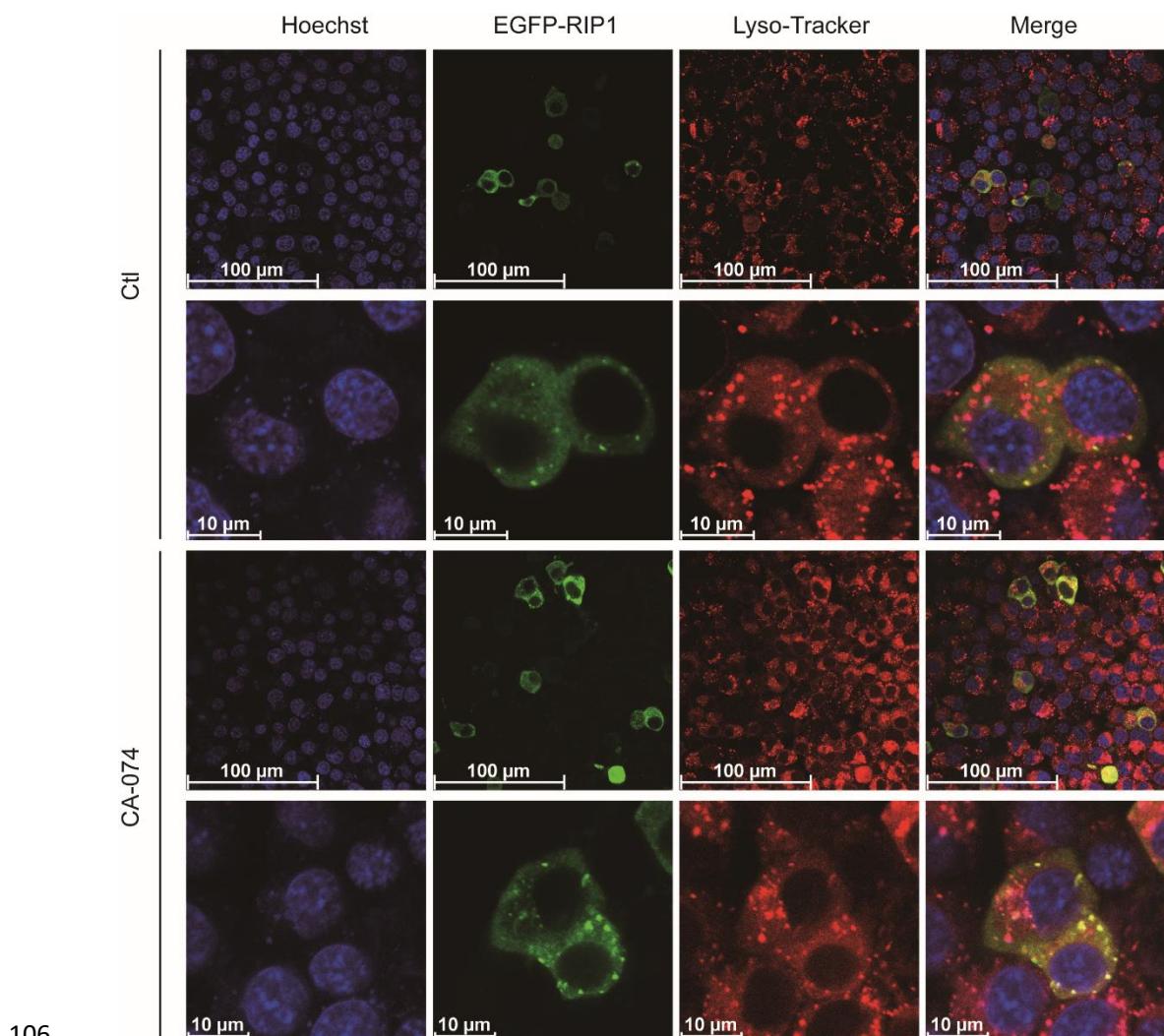
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105 **Figure S4**



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107 **Fig. S4. RIP1 is degraded in lysosomes.**

108 Confocal micrographs of Neuro-2a transfected with PCMV-C-EGFP-RIP1 and stained with
109 Hoechst (blue, DNA) and Lyso-Tracker Red after CA-074 (10 μ M) stimulation for 12 h. Figures
110 are representative of three independent experiments.

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118 **Table S1. sgRNA used for the construction of knockout cell lines.**

sgRNA	Sequences
Mouse HDAC1 sgRNA-F	CACCG CTTCAAGTCAATGTTGGTG
Mouse HDAC1 sgRNA-R	AAAC CACCAACATTGACTGAAAG C
Mouse HDAC2 sgRNA-F	CACCG CCATGAAGCCTCATAGAAC
Mouse HDAC2 sgRNA-R	AAAC GATTCTATGAGGCTTCATGG C
Mouse HDAC3 sgRNA-F	CACCG CCCAATGAAACCTCATGCC
Mouse HDAC3 sgRNA-R	AAAC GGCGATGAGGTTTCATTGGG C
Mouse HDAC8 sgRNA-F	CACCG GATGGCCTCATCACCTTCT
Mouse HDAC8 sgRNA-R	AAAC AGAAGGTGATGAGGACCATC C

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120 **Table S2. Primers used for Polymerase Chain Reaction in this paper.**

Primers	Sequences
Mouse IL-1 β forward primer	GAAATGCCACCTTTGACAGTG
Mouse IL-1 β reverse primer	TGGATGCTCTCATCAGGACAG
Mouse Mcp1 forward primer	TTAAAAACCTGGATCGAACCAA
Mouse Mcp1 reverse primer	GCATTAGCTTCAGATTACGGGT
Mouse Mip2 forward primer	CCAACCACCAGGCTACAGG
Mouse Mip2 reverse primer	GCGTCACACTCAAGCTCTG
Mouse Cox2 forward primer	TTCAACACACTCTATCACTGGC
Mouse Cox2 reverse primer	AGAAGCGTTGCGGTACTCAT
Mouse β -ACTIN forward primer	GGCTGTATTCCCTCCATCG
Mouse β -ACTIN reverse primer	CCAGTTGGTAACAATGCCATGT
Mouse Ctsa forward primer	CCCTCTTCCGGCAATACTCC
Mouse Ctsa reverse primer	CGGGGCTGTTCTTGGGTC
Mouse Ctsb forward primer	TCCTTGATCCTCTTCTTGTGCC
Mouse Ctsb reverse primer	ACAGTGCCACACAGCTCTTC
Mouse Ctsc forward primer	CAACTGCACCTACCCTGATCT
Mouse Ctsc reverse primer	TAAAATGCCCGGAATTGCCCA
Mouse Ctsd forward primer	GCTTCCGGTCTTGACAACCT
Mouse Ctsd reverse primer	CACCAAGCATTAGTTCTCCTCC
Mouse Ctse forward primer	GACATCAGTCCCTCGGAAGA
Mouse Ctse reverse primer	AGGGGTTCATTGACACTCGAATA
Mouse Ctsf forward primer	CCCTGGAAAGCCACACTAGAG
Mouse Ctsf reverse primer	GGGCTACAGTCCCTCCTCAG
Mouse Ctsg forward primer	AGGGTTCTGGTGCAGAGAAG
Mouse Ctsg reverse primer	GTTCTGCGGATTGTAATCAGGAT
Mouse Ctsh forward primer	ACCGTGAACGCCATAGAAAAG
Mouse Ctsh reverse primer	TGAGCAATTCTGAGGCTCTGA
Mouse Ctsk forward primer	GAAGAAGACTCACCAGAAGCAG
Mouse Ctsk reverse primer	TCCAGGTTATGGGCAGAGATT
Mouse Ctsl forward primer	ATCAAACCTTAGTGCAGAGTGG

Mouse Ctsl reverse primer	CTGTATTCCCCGTTGTGTAGC
Mouse Cts0 forward primer	CAGCGTGGTGAGTGCCATAG
Mouse Cts0 reverse primer	ACCGAGGCAGCCAGAATTATTA
Mouse Ctss forward primer	CCAITGGGATCTCTGGAAGAAAA
Mouse Ctss reverse primer	TCATGCCCACTTGGTAGGTAT
Mouse Ctsw forward primer	TGACTCCCTCCTCACCAAGG
Mouse Ctsw reverse primer	GCTGGGTTCCAGTAACCTCG
Mouse Ctsz forward primer	GGCCAGACTTGCTACCATCC
Mouse Ctsz reverse primer	ACACCGTTCACATTCTCCAG